MACROAUTORADIOGRAPHIC STUDY OF THE DISTRIBUTION OF ^<14>C-DIMETHYLNITROSAMINE IN MICE AND FETUSES

AUTHOR(S):

TAKAHASHI, Gonya

MACROAUTORADIOGRAPHIC STUDY OF THE DISTRIBUTION OF ^{14}C-DIMETHYLNITROSAMINE IN MICE AND FETUSES

Gonya TAKAHASHI

Department of Pathology, Chest Disease Research Institute, Kyoto University, Kyoto

Since Magee et al[1] reported that hepatic cancer was induced by dimethylnitrosamine (DMN) in the rat, nitroso compounds have demonstrated potent carcinogenic activity in various organs of some species[2,3]. DMN induces tumors in the lung of mice in high incidence[4] and sometimes in the liver and kidney of mice[4,5].

On the other hand, Mohr et al.[6] reported lung adenoma induction in offspring of mice whose mothers had been injected with diethylnitrosamine (DEN).

In the present investigation, the distribution of ^{14}C-labeled DMN in mice and their fetuses was demonstrated using macroautoradiography to elucidate the tissue affinity of this carcinogen.

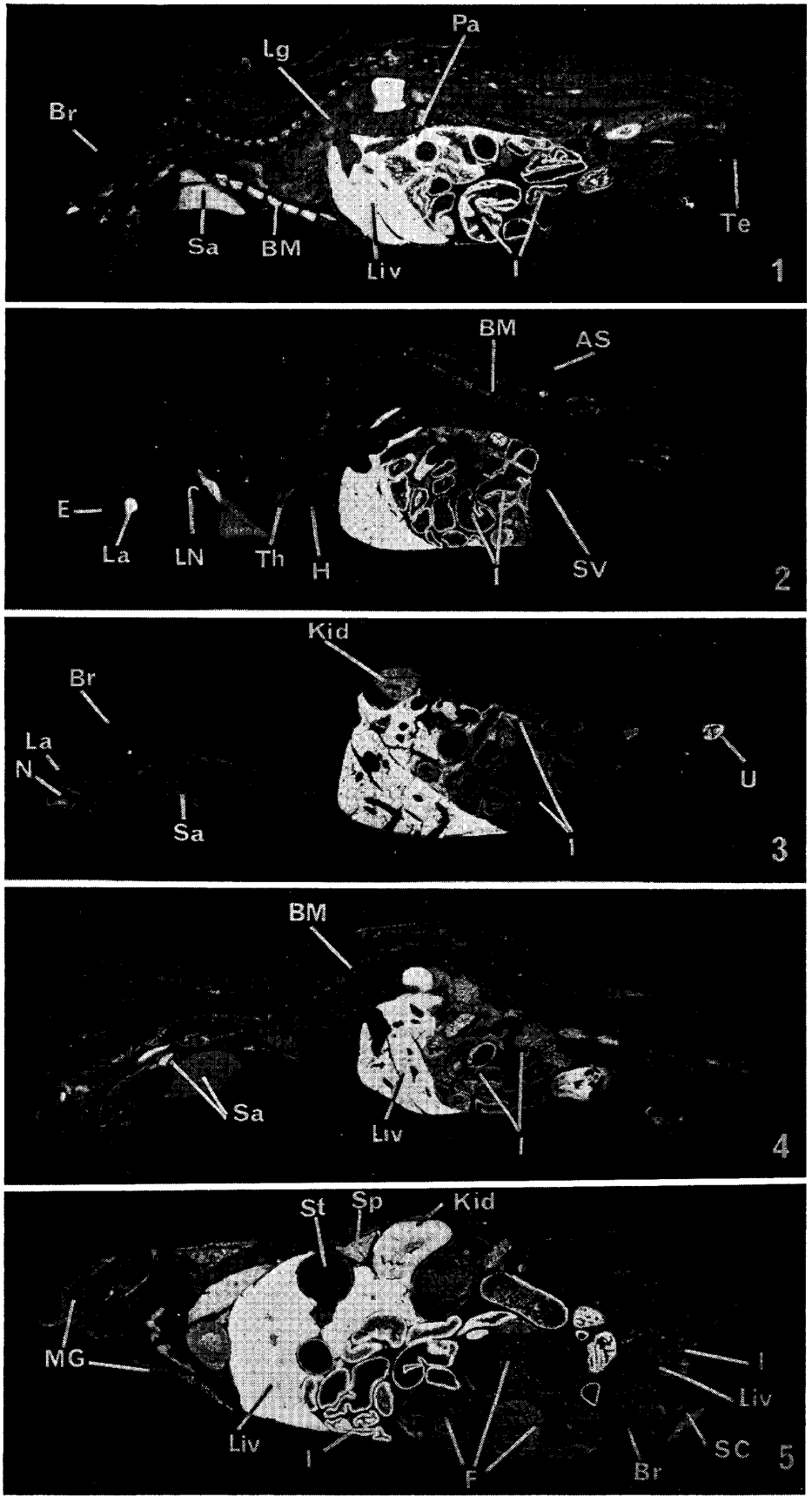
MATERIALS AND METHODS

^{14}C-labeled DMN (4.5 mCi/mmole) was obtained from New England Nuclear (Boston, Mass). Three female ICR mice were housed with a male overnight and the 0 day of pregnancy was taken to be the next morning when a vaginal plug was found.

Five μCi of ^{14}C-DMN were dissolved in water and this one dose was injected intravenously or given by gastric intubation to adult ICR mice, and to pregnant mice on the 18th day of gestation. These mice were sacrificed with ether at various intervals, and immediately frozen in acetone-dry ice mixture. Whole body saggital sections of the frozen mouse were prepared at $-12°C$ according to the methods described previously[7]. Each serial section was 100 μm thick. The sections were exposed to Sakura industrial type N X-ray film for 1 to 2 weeks.

RESULTS

Thirty minutes after intravenous administration, the radioactivity derived from ^{14}C-labeled DMN was found mostly in the liver, kidney, bone marrow, the salivary, lachrymal and mammary glands, and appendage of the skin, pancreas, mucous membranes of the nasal cavity, intestine, and glandular stomach, with less in the lymph nodes, spleen and lungs. The radioactivity was minimal in the nervous tissues, muscle, testes and contents of the alimentary tract. The pattern of distribution was the same as long as 2 hours later. The radioactivity observed in the urinary and gall bladder suggested that this chemical is to some extent excreted into the urine and bile.


Pa
Lg
Br
Sa
BM
Liv
I
Te
1
BM
AS
E
La
LN
Th
H
I
SV
2
Kid
Br
La
N
Sa
I
U
3
BM
Sa
Liv
I
4
St
Sp
Kid
MG
Liv
I
F
Br
SC
I
Liv
5

Radioactivity was observed in the lower part of the intestinal tract as early as 30 min after administration, suggesting that radioactive substances are excreted into the intestinal tract via the mucous membrane.

Thirty minutes after gastric intubation, the radioactive substances were absorbed rapidly from the alimentary tract and distributed most intensely in the liver, kidney, spleen and pancreas. The radioactivity was also intense in the glands but low in the lung, nervous tissues, muscle, testes and bone marrow. Two hours later, the pattern of the density of radioactivity on film was relatively the same as observed at 30 min, except for the bone marrow whose radioactivity was particularly intense 2 hours later.

^{14}C-labeled DMN or its radioactive metabolites passed easily through the placenta and were found in fetal organs. The relative concentration of radioactivity was rather intense in the fetal liver, intestinal wall, bone marrow and thymus, but in almost all organs the labeling was distributed uniformly.

DISCUSSION

The present study shows that radioactivity derived from ^{14}C-labeled DMN is distributed in various organs especially the liver, kidney, mucous membranes of the alimentary tract and nasal cavity, the salivary, lachrymal and mammary glands and appendage of the skin. The intensity of the radioactivity in the liver and kidney of mice is in accord with previous reports of the induction of liver and kidney tumor in mice by this carcinogen[4,5]. The appearance of radioactivity in glandular tissues is of further interest since tumors of the nasal sinus have been induced in rats after inhalation of DMN[8] and in mice by subcutaneous asministration of DEM[9]. The glandular stomach is susceptible to N-nitroso-N-methyl-urethane[10] or N-methyl-N′-nitro-N-nitrosoguanidine[11] in rats.

Certain nitroso compounds such as methylnitrosourea[12] and N-methyl-N′-nitro-N-nitrosoguanidine[13] tend to be distributed intensely in nervous tissues in the rat and mouse respectively, but DMN has no such tendency as demonstrated herein.

The radioactive substances passed across the placenta and were distributed among various fetal organs. Although transplacental carcinogenesis has not been demonstrated with this carcinogen in mice, DEN is known to be carcinogenic by the transplacental route in mice[6]. and DEN in rats[14] in low incidence and methyl or ethylnitrosourea[15][16], or nitrosomethylurethane[17] are also carcinogenic via the same route in mice or rats.

It is known that DMN is demethylated rapidly in vivo and the biochemical lesion is produced by its metabolites but not by DMN itself[18] and that there are large strain differences in mice and species differences in response to particular nitroso compound[2,3]. Therefore, it has

Macroautoradiography of sagittal sections of mice administered ^{14}C-labeled DMN.

Figs. 1 and 2: Thirty minutes and 2 hours after intravenous administration; **Figs. 3 and 4**: Thirty minutes and 2 hours after oral administration; **Fig. 5** : One hour after intravenous administration in a pregnant mouse on the 18th day of gestation. Abbreviations: AS; appendage of the skin, BM; bone marrow, Br; brain, E; eye F; fetus, H; heart, I; intestine, Kid; kidney, La; lachrymal glands, Lg; lung, Liv; liver, LN; lymphnode, MG; mammary gland, N; nasal gland, Pa; pancreas, Sa; salivary gland, SC; spinal cord, Sp; spleen, St; stomach, SV; seminal vesicle, Te; testis, Th; thymus, U; urinary bladder.

less meaning to compare the tissue susceptibility to DMN with the distribution of radioactivity in the tissues.

Any way, the macroautoradiograms demonstrated herein showed the distribution of radioactivity in small parts of certain organs and bone marrow, where the concentration of radioactivity is not easily measured by conventional chemical methods.

SUMMARY

Distribution of ^{14}C-labeled dimethylnitrosamine administered orally or intravenously to adult or pregnant mice was studied by macroautoradiography. The radioactivity was localized in the liver, kidney, bone marrow and some glandular organs e.g., the mucous membranes of the alimentary tract and nasal cavity, the salivary, lachrymal and mammary glands and appendage of the skin, with less in the lymphnodes, spleen and lungs, and was minimal in the nervous tissues, muscle and testes. The pattern of the distribution of radioactivity in tissues was very similar with both routes of administration. The radioactive substances passed readily across the placenta and was distributed uniformly in fetal organs.

REFERENCES

1) Magee, P. N., Barnes, J. M.: The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine. Brit. J. Cancer, 10: 114, 1956.
2) Druckrey, H., Preussmann, R., Ivankovic, S., Schmähl, D.: Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten. Z. Krebsforsch. 69: 103, 1967.
3) Magee, P. N., Barnes, J. M.: Carcinogenic nitroso compounds. Advan. Cancer Res., 10: 163, 1967.
4) Toth, B., Magee, P. N., Shubik, P.: Carcinogenesis study with dimethylnitrosamine administered orally to adult and subcutaneously to newborn BALB/C mice. Cancer Res., 24: 1712, 1964.
5) Takayama, S., Oota, K.: Malignant tumors induced in mice fed with N-nitrosodimethylamine. Gann, 54: 465, 1963.
6) Mohr, V., Althoff, J.: Die diaplacentare Wirkung des Cancerogens Diäthylnitrosamine bei der Maus. Z. Krebsforsch 67: 152, 1965.
7) Takahashi, G. Yasuhira, K.: Macroautoradiographic and radiometric studies on the distribution of 3-methylcholanthrene in mice and their fêtuses. Cancer Res., 33: 23, 1973.
8) Druckrey, H., Steinhoff, D., Preussmann, R., Ivankovic, S.: Erzeugung von Krebs durch eine einmalige Dosis von Methylnitroso-Harnstoff und verschiedenen Dialkylnitrosamine an Ratten. Z. Krebsforsch. 66: 1, 1964.
9) Hoffmann, F., Graffi, A.: Nasenhohlentumoren bei Mausen nach precutaner Diäthylnitrosaminapplikation. Arch. Geschwülstforsch. 23: 274, 1964.
10) Schoental, R.: Induction of tumors of the stomach in rats and mice by N-nitroso-N-alkylurethanes. Nature, 199: 190, 1963.
11) Sugimura, T., Fujimura, S.: Tumor production in glandular stomach by N-methyl-N′-nitro-N-nitrosoguanidine. Nature, 216: 943, 1967.
12) Kleihues, P., Patzschke, K.: Verteilung von N-[^{14}C]-methyl-N-nitroharnstoff in der Ratte nach systemischer Applikation. Z. Krebsforsch, 75: 193, 1971.
13) Takahashi, G.: Selective distribution of the radioactivity in nerve tissues of the mouse after intravenous administration of ^{14}C-N-methyl-N′-nitro-N-nitrosoguanidine. Gann, 65: 363, 1974.
14) Alexandrov, V. A.: Blastomogenic effect of dimethylnitrosamine on pregnant rats and their offspring. Nature, 218 280, 1968.
15) Rice, J. M.: Transplacental carcinogenesis in mice by 1-ethyl-1-nitrosourea. Ann. N. Y. Acad. Sci., 163, 813, 1969.
16) Wechsler, W.: Carcinogenic and teratogenic effects of ethylnitrosourea and methylnitrosourea during

pregnancy in experimental rats. I.A.R.C. Scientific Publication No. 4: 127, 1973.
17) Tanaka, T.: Transplacental induction of tumors and malformations in rats treated with some chemical carcinogens. I.A.R.C. Scientific Publication No. 4: 100, 1973.
18) Dutton, A. H., Heath, D. F.: Demethylation of dimethylnitrosamine in rats and mice. Nature, 178: 644, 1956.